There is No High Output Without High Throughput

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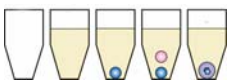
Introduction

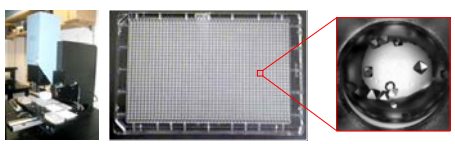
The HTS laboratory was established at HWI in February 2000. Since that time 7.5 million crystallization experiments have been set up using this facility for both the structural genomics (NESG, SGPP) and structural biology communities (> 4800 samples). This poster describes improvements to the laboratory infrastructure, the success rate for determining crystallization conditions, and HTP efforts to develop effective crystallization screens for transmembrane proteins.

Overview

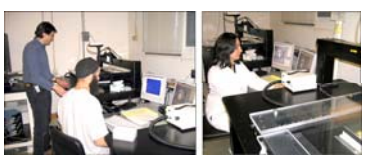
Samples arrive and information is entered in the HTS lab database

Microbatch-under-oil Crystallization (1): **Oil** is used to encapsulate an experiment drop containing crystallization cocktail and a macromolecular sample.





Tango LHS are used to set up 1536 unique microbatch under oil experiments for each sample in a single microassay plate (2).

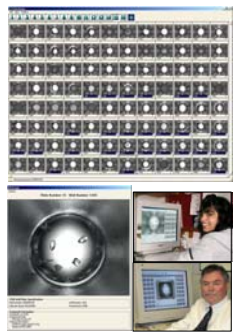


Completed experiment plates are imaged over a 4 week time course. The HTS lab database creates a table of read dates and emails the operator when a read is not completed as scheduled.

Computer and Database Infrastructure

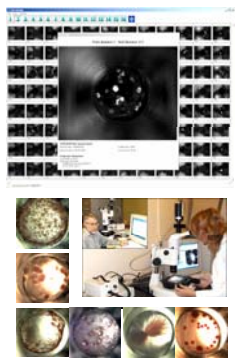
A highly redundant computer infrastructure has been developed to control all aspects of the HTS lab. The infrastructure was designed to eliminate interruption of data collection and to safeguard data after it is collected. A database controls and tracks sample information and automates tasks. This includes email notification at the completion of each plate read notifying collaborators that image data is available through a secure ftp server.

Images used to record the outcome of experiments are manually reviewed using MacroScope software. Images that contain crystals are marked and the file saved. Classified images (crystals / no crystals) are forwarded to crystal optimization and image analysis groups. These images show results from the Hampton Research Index Screen on sample P000003249.



Crystal Detection and Verification

Greiner BioOne has developed a low birefringence 1536 well plate to aid in the identification of crystalline outcomes. Crystal composition (salt or protein) is verified by adding dye to the experiment plates. Software takes an output file from MacroScope and controls a videomicroscope equipped with a translation stage. Wells containing crystals are positioned for inspection.



References

(1) Chayen, N.E., Stewart, Patrick D. Shaw, Blow, David M., *Microbatch crystallization under oil — a new technique allowing many small-volume crystallization trials.* Journal of Crystal Growth, 1992. **122**(1-4): p. 176-180.

(2) Luft, J.R., Collins, R.J., Fehrman, N.A., Lauricella, A.M., Veatch, C.K., and DeTitta, G.T., *A deliberate approach to screening for initial crystallization conditions of biological macromolecules.* Journal of Structural Biology, 2003. **142**(1): p. 170-9.

(3) Wiener, M.C. and Snook, C.F., *The development of membrane protein crystallization screens based upon detergent solution properties.* J. Cryst. Growth, 2001. **232**: p. 426-431.

(4) Carter, C.W., Jr., *Response surface methods for optimizing and improving reproducibility of crystal growth*, in *Methods in Enzymology*, Carter, C.W., Jr. and Sweet, R.M., Editors. 1997, Academic Press: San Diego. p. 74-99.

Acknowledgements

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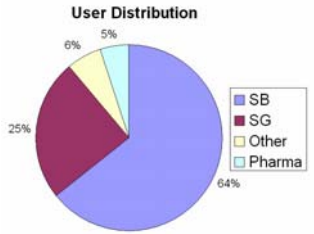
Success Rate for the Structural Biology Community

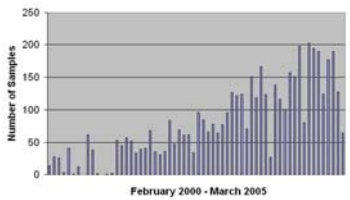
87 randomly selected samples between June – August 2004
One or more likely macromolecular crystalline outcome = success

52% (45/87) of the samples produced a crystalline outcome
40% (18/45) of the samples produced **5 or fewer hits (1536 trials)**

Throughput and Sample Sources

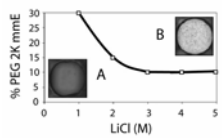


Number of Samples per Month



Developing Crystallization Screens for TMPs.

Detergent phase boundaries are being measured with organic polymers and salts used for TMP crystallization using a systematic sampling of the precipitant landscape (3).



Data is being collected to profile a phase separation diagram for each detergent under study. Crystallization space is refined from these data using response surface techniques (4). Using the HTP infrastructure it is possible to measure the phase behavior for as many as 12,000 combinations of detergents/precipitants in a single day.

To collaborate with us:
http://www.hwi.buffalo.edu

Click on the crystal.

Images that form the border are a portion of the 228 crystal hits from sample P000003249, a sample submitted by a collaborator from the structural biology community.